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REVIEW

Understanding Aspects of Aluminum Exposure in Alzheimer's Disease Development

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Abstract

Aluminum is a ubiquitously abundant nonessential element. Aluminum has been associated with neurodegenerative diseases such as Alzheimer's disease (AD), amyotrophic lateral sclerosis, and dialysis encephalopathy. Many continue to regard aluminum as controversial although increasing evidence supports the implications of aluminum in the pathogenesis of AD. Aluminum causes the accumulation of tau protein and Aβ protein in the brain of experimental animals. Aluminum induces neuronal apoptosis *in vivo* and *in vitro*, either by endoplasmic stress from the unfolded protein response, by mitochondrial dysfunction, or a combination of them. Some, people who are exposed chronically to aluminum, either from through water and/or food, have not shown any AD pathology, apparently because their gastrointestinal barrier is more effective. This article is written keeping in mind mechanisms of action of aluminum neurotoxicity with respect to AD.

INTRODUCTION

Aluminum (Al) is the third most abundant element which comprises about 8% of earth's crust, after oxygen and silicon. Al is too reactive chemically to occur as a free metal in nature. Instead, it always occurs in combination with other elements such as hydroxide, silicate, sulfate, and phosphate. The wide distribution of this element ensures the potential for causing human exposure. Aluminum is used in the production of every-day products such as cookware is made from aluminum, soda cans, aluminum foil, antacids, aspirin, vaccines, and flour (33).

It has been suggested that there is a relationship between chronic routine exposure to Al and increased risk of a number of neurodegenerative disorders including Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), and AD-type dementia in Parkinson patients (9).

Failure of amyloid trials has caused many researchers to be more critical of current dogma. Recent studies have suggested that neuroinflammation and neuronal loss precede amyloid-β plaque deposition in the hAβPP-J20 mouse model of AD (158), The earliest damage in humans with AD occurs as neurofibrillary tangles (NFTs) in the entorhinal cortex cells of origin for the performant pathway. Neurofibrillary tangle formation involves aluminum interactions with hyperphosphorylated tau (147). AD is one of the prevalent neurodegenerative diseases affecting more than five million people in the United States and is projected to affect more than quadruple that amount during the next 50 years if the present situation persists (130). AD is the most common cause of dementia in the elderly. The well-defined lesions in AD pathology are extracellular deposition of the amyloid-β (Aβ) protein, intracellular NFTs, and selective neuronal loss. (Although the cause of AD remains poorly understood, multiple factors are reported to influence AD onset. Mounting genetic and biochemical data support the hypothesis that amyloid-β protein (Aβ) accumulation and aggregation in the brain are early and central events in the pathogenesis of AD.) The primary factor, among the multiple factors in the pathogenesis of early onset AD, is mutations in amyloid precursor protein (AβPP) and presenilins 1 and 2, that lead to an increase in the production of $A\beta_{42}$ (14). The ϵ4 allele of apolipo protein E (Apo E) is the most prevalent risk factor linked to AD. Additionally linked to AD are increased levels of homocysteine, cholesterol, and several minor metal ions such as Al, iron, and copper. Dollken *et al* (1897) first reported Al neurotoxicity in experimental animals and stated that free radical formation is due to Al. Klatzo *et al* (1965) reported that injections of Al salts into rabbit brain led to the formation of NFTs, one of the hallmarks of AD. It has been shown that rabbits may provide a unique animal system

for producing neurofibrillary pathology because rabbits belong to the mammalian order lagomorpha which more closely resembles primates than rodents (52). Crapper *et al* (1973) reported neurofibrillary degeneration in cats with Al accumulation. It has also been shown that Al salts injected intracerebrally or peripherally in rabbits, mice, cats, and monkeys induce the formation of neurofibrillary threads (6, 11, 12, 22, 23, 25, 45, 52, 77, 142). Al has been shown to accumulate to different extents in a number of regions of rat brain, in particular the hippocampus, which is the site of memory and learning (24, 26, 66, 71). Becaria *et al* (2002) (described Al as a toxicant when present in high doses in circulation and) suggested that a prolonged exposure to relatively low levels of dietary Al may be responsible for the observed neurotoxic effects. Al has been detected in both senile plaques and NFT-bearing neurons in the brains of AD patients (McLachlan *et al* 1996), which suggests roles for this metal in AD. Earlier studies from our laboratory have also shown that acute as well as subacute exposure of rats to Al causes marked reduction in acetyl choline esterase activity (66, 112) and significant deterioration in cognitive functions of rats (148), which are symptoms of AD. Walton (2006) showed that Al was present in the hippocampal neurons of AD patients and hence Al might be playing a role in the formation of NFTs (145). Miu *et al* (2003) reported the effects of long term Al exposure in terms of behavioral and brain modifications (98). They observed numerous ghost-like neurons with cytoplasmic and nuclear vacuolations with Al deposits. Their study also reported hippocampus containing extracellular accumulations of Al and amyloid surrounded by nuclei of degenerating cells. The aforementioned modifications are reminiscent of those observed in AD. Perl and Moalem (2006) also presented evidence that Al, a highly reactive element known to crosslink hyperphosphorylated proteins, may play an active role in the pathogenesis of critical neuropathological lesions in AD and the other related disorders. Al binding can enhance Aβ protein penetration of the blood brain barrier (5). Several studies have demonstrated that Al can induce pathological changes similar to AD including the accumulation of tau and Aβ, neuronal apoptosis, conformational changes of Aβ and disease related proteins, and disruption of iron and calcium homeostasis (16, 96, 155, 162, 163, 166, 167). Kawahara (2005) demonstrated that Al can cause the accumulation of tau protein and Aβ protein in experimental animals leading to induced neural apoptosis *in vivo* as well as *in vitro* (72). Studies show increased oxidative damage, neuronal degeneration, and hyperphosphorylated tau in rat model of AD, providing further evidence that Al may play a role AD (145, 146). In this article, we review material in favor of, and against, the concept of Al neurotoxicity in the context of AD.

AL EXPOSURE AND ASSESSMENT

Al has been used for many years in shipbuilding, petroleum processing, rubber industry, medicine, food processing, agriculture, and treatment of water. Various pathological conditions, such as asthma-like disorders and pulmonary fibrosis, have been reported among workers involved in the Al industry (103). The principal routes of human exposure to Al are by ingestion of food or water containing Al and the inhalation of Al as fine particles (118, 120, 121, 143, 163). The average human Al consumption through food and beverages (excluding drinking water) ranges from 2.5 to 13 mg/day and varies with composition of the food eaten, country of residence, age, and sex. In the US, the total dietary Al intake estimates for adult men and women are 8–9 and 7 mg/day (These Al values are appropriate for fresh foods but are too low for commercially prepared (processed) foods. Given figures are higher but more realistic considering one piece of sliced individually wrapped cheese can contain), respectively (44, 53, 157). The metal can be taken into the body from food, by both naturally occurring Al and artificial dietary additions from sources such as additives, water, cooking utensils, containers (including Al drink cans), aerosols, and dusts. Although it is well known that less than 1% of ingested Al is absorbed, significant absorption and retention can occur either (i) when long-term intake exceeds 1000 mg/day or (ii) when chronic aluminum exposures at the high end of the human range for dietary aluminum levels (around 100 mg/day) (149).

Even though bioavailability of Al from water (0.3%) is higher than from food (0.1%), due to differences in daily Al intake, drinking water contributes (considerably less) to the overall human exposure to Al (38, 143). The issue of safe levels of Al in drinking water is of considerable interest to public health officials and regulatory agencies because Al is widely used as a coagulant in water treatment (92).

Al intake from drinking water

The World Health Organization (157) has recommended that Al concentrations in drinking water not to exceed 0.2 mg/L, stating "...the positive relationship between Al in drinking water and AD, which was demonstrated in several epidemiological studies, cannot be totally dismissed. Taken together, the relative risks for AD from exposure to Al in drinking water above 100 μg/liter are low (less than 2.0) (67)." The US Environmental Protection Agency set 0.05 mg/L as a limit in 1985. The European Economic Community has established a recommended guideline of 0.05 mg/L and a maximum permissible level of 0.2 mg/L (38). Numerous epidemiological reports were published on the subject of exposure to Al from drinking water and risk of AD (38).

First attempts to relate the actual levels of Al in drinking water to AD were made in 1986, when two parallel Norwegian studies reported higher mortality from dementia (from all causes) in areas with high concentrations of Al in drinking water (38, 143). A cross-sectional investigation (90) conducted a few years later in England and Wales found that among all other causes of dementia the risk of AD was 1.5 times higher in districts where the mean Al concentration in drinking water exceeded 0.11 mg/L, compared to the districts where Al concentrations were less than 0.01 mg/L; no relation was found between any other neurological condition and concentrations of Al in water (90). Another ecological study, conducted in Newfoundland, Canada, found an excess of dementia mortality (confirmed by death certificates) from the North shore of Bonavista Bay in 1985 and 1986, a phenomenon that could not be explained by differences in sex, age, or other parameters (43). That specific area of the bay was known to have high Al concentration in the drinking water (165 mg/L) and low pH (5.2). A case-control study conducted in Ontario, Canada, used hospital discharge data with a diagnosis of dementia or presenile dementia to describe a dose-response relationship between the Al content of drinking water and risk of AD (106). The relative risks associated with the consumption of drinking water containing Al concentrations of <0.01, 0.01–0.1, 0.1–0.199, and >0.200 mg/L were estimated to

be 1.00, 1.13, 1.26, and 1.46, respectively. Subsequent reanalysis of the data confirmed a stronger dose-response relationship for those over 75 years of age (39, 40, 84, 103). No additional adjustments for confounding factors except for age and sex were made in this study. Another Canadian investigation was done by Forbes *et al* (1994, 1995), who studied the relationship between Al, fluoride, and other constituents in drinking water and cognitive function. The research project was based on the Ontario Longitudinal Study of Aging, where 2000 men have been followed for about 30 years. Analysis of the data showed that men living in areas with high Al and low fluoride concentrations in drinking water were about three times more likely to have some form of mental impairment than those individuals living in the areas with low Al and high fluoride levels. Further analysis suggested that at neutral pH, relatively low Al concentrations, and relatively high fluoride concentrations in drinking water decreased the odds of cognitive impairment by a factor of five (39, 40). A case-control study on autopsy-verified material conducted by McLachlan *et al* (1996) compared the AD patients and controls without brain pathology on the basis of neuropathologic criteria. Al concentration in drinking water at last residence before death was used as the measure of exposure (96). The authors reported elevated risks for histopathologically verified AD to be associated with higher levels of Al (odds ratio (OR) = 1.7, 95% CI 1.2–2.5, for a level of Al >0.1 mg/L). The authors later obtained even larger estimates (OR of 2.5 or greater) and implemented a 10-year weighted residential history in the analysis. At an Al concentration of 0.125 mg/L in drinking water, the OR for risk of AD was 3.6 (95% CI 1.4–9.9), at 0.150 mg/L the OR was 4.4 (95% CI 0.98–20), and at 0.175 mg/L it was 7.6 (95% CI 0.98–61). The study had several methodological strengths including the diagnostic quality of the data (96).

In France, Rondeau *et al* (2000) utilized the data from a large prospective Paquid cohort study, which followed 3777 subjects, aged 65 years and older, for up to 8 years, and recorded all new cases of dementia and AD (121). In each residential area the range and mean exposure to Al (0.001–0.459 mg/L, median 0.009 mg/L) and silica (4.2–22.4 mg/L) from drinking water were recorded. The analysis of data adjusted for age, gender, educational level, place of residence, and wine consumption revealed that the risk of dementia was higher for individuals who lived in areas with high levels of Al in water (mean concentration >0.1 mg/L) compared with people residing in areas with Al levels less than 0.1 mg/L (relative risk 1.99, 95% CI 1.20–3.28, $P = 0.007$). Higher silica concentrations (>11.25 mg/L), adjusted for age and gender, were associated with a reduced risk of dementia (RR = 0.71, 95% CI 0.56–0.91, $P = 0.007$). The adjusted relative risk of AD for individuals exposed to drinking water with Al concentration > 0.10 mg/L was 2.14 (95% CI 1.21–3.80, $P = 0.007$). As with dementia, the risk of AD was reduced in the presence of high concentrations of silica (RR = 0.73, 95% CI 0.55–0.99, $P = 0.04$). Although no dose-response effect was found, the authors concluded that a concentration of Al in drinking water above 0.1 mg/L may be a risk factor for dementia and AD.

In addition, intriguing findings by Exley and Esiri (2006) have provided additional support for the Al-AD hypothesis (34). The authors reported a rapidly progressive, fatal neurological illness a 58-year-old woman who, at autopsy, showed dramatic Aβ deposition of cerebral cortical and leptomeningeal blood vessels, modest numbers of NFTs and Lewy bodies, and evidence of very high Al content in affected brain regions (34). The deceased was amongst the 20,000 people who were accidentally exposed to exceedingly high concentrations of Al (approximately 500–3000 times the acceptable limit of 0.200 mg/L) in their water supplies in July 1988. Although this case alone cannot confirm the causative role of Al in the development of AD, it is certainly the first autopsy-documented case of AD-like condition potentially linked to Al exposure from drinking water, which warrants further investigations and surveillance of all affected individuals (37).

Several studies have purported to show lack of significant difference in AD in human populations exposed to high vs. low aluminum levels in their drinking water. Wettstein *et al* (1991) compared the cognitive skills between two groups of senile long-term residents of Zurich. One group of subjects lived in an area with high Al concentration in drinking water (mean concentration <0.10 mg/L); the other group lived in the area with relatively low Al levels (mean <0.01 mg/L). The authors did not find any substantial difference in cognitive impairment between the two groups. However, the significance of these negative results might be limited by the fact that the study only tested for cognitive impairment of any kind and relied on only two sources of drinking water (151). Thus the bioavailability of Al was likely to be different in the two water sources. Likewise, Martyn *et al* (1997) found no association between the prevalence of AD and higher Al concentrations in drinking water when comparing 106 men with early onset AD (91). Also, it should be noted that this study was based on patients with early-onset AD who are more affected by their genetic constitution than patients with sporadic AD. These results were similar to results of Forster DP *et al* (1995) whose study was also based on early-onset AD patients. Early-onset AD patients are more likely to contain mutations in their AbetaPP and/or presenilin genes, so they are differ from patients with sporadic AD by being more affected by their genetic constitution rather than by environmental influences in AD. The preponderance of these epidemiological studies has shown a positive relationship between risk for sporadic AD and levels of aluminum in public drinking water supplies.

Al intake from diet (epidemiological studies)

Even though most epidemiological studies have focused Al exposure from drinking water, Al exposure from food consumption is typically 10-fold or greater than exposure from drinking water (120). Al in the food supply comes from natural sources, water used in food preparation, and food additives. Depending on several variables, the Al content of food can vary greatly and, in certain vegetables, Al may be adhering to the vegetable through the soil.

Dietary Al toxicity in healthy individuals in relation to AD has not been reported, primarily because of the difficulties, or even potential impossibility, for accurately assessing chronic dietary Al exposure, particularly where the subjects include AD patients. One pilot study conducted by Rogers and Simon (1999) in a geriatric center, employed 23 case-control pairs. Close relatives were interviewed about dietary intake of the cases, with special attention to food high in Al such as American cheese, chocolate pudding, doughnuts, pancakes, or muffins. In the same study, resulting ORs were unstable and not statistically significant, except for one food category that contained waffles, pancakes, biscuits, muffins, corn bread or corn tortillas. Although interesting, this pilot study strongly suggested the need for additional confirmatory studies

with larger sample size and more rigorous design. Other sources of dietary Al include tea, which contains particularly high levels of Al (300–2677 mg/kg), and may contribute up to 50% of the total daily Al intake in the countries with high tea consumption and small intake of Al from other sources (111, 156). Yet, several studies that explored the link between tea consumption and risk of AD were unsuccessful in providing evidence to support this hypothesis.

In a case-control study comparing cases of clinically diagnosed presenile dementia of the Alzheimer's type and controls matched for age and sex, exposure to Al from tea was not a significant risk factor for dementia (42). Similarly, no relationship was found for exposure to Al from drinking water or medicinal sources in this investigation. A case control study completed in Canada produced a similar OR for the risk of AD; however, the serving amount of tea served was not specified (42).

Some foods, known as Al accumulators (eg, herbs and tea leaves) may naturally contain more than 5 mg/g of this metal. Intake of dietary Al is higher in the United States than in other countries, due to wide use of Al-containing food additives (120, 143). A previously mentioned study by Rogers and Simon (1999) also found no significant relation between tea consumption and AD, which the authors explained by a possible loss of Al during the tea processing and binding of Al to organic compounds, possibly lemon juice. Thus, despite relatively high Al content and high dietary consumption, the role of tea in development of AD or similar pathologies remains controversial (120). Overall, the results of epidemiological investigations are promising and suggest an association between chronic exposure to Al and risk of AD. Although, to prove this hypothesis, meta-analysis studies are needed. The significant contribution of food to Al exposure may mean that an omitted-variable bias has been present in many previous studies that have only focused on Al in water and, if this is true, these studies might have significantly underestimated the relationship of Al to AD.

Recently Molloy *et al* (2007), conducted a double-blind, randomized, controlled study. The authors could not find any significant differences in neuropsychological test scores after brief exposure to high Al ingestion (150 μg/L) in normal volunteers or in patients with cognitive impairment (101). The results did not support the hypothesis that Al ingested at these doses produces acute effects on cognition or adverse effects, nor did they reveal that AD patients are more vulnerable to such outcomes in the short-term. It is possible that higher doses of Al and citrate over a much longer exposure period might produce subacute neuropsychological effects. The Al doses used resulted in serum Al levels exceeding presumed threshold values for neurotoxicity, and in the absence of measuring data to the contrary, the use of higher doses of Al or longer exposure of humans to neurotoxic serum Al levels in future studies would be limited by ethical considerations about possible long term implications of such exposures.

Al neurotoxicity—*in vitro* studies and *in vivo* studies (in experimental animals)

Hewitt *et al* (1991) explanted cores of subaqueductal midbrain nuclei and maintained on thin cubes of Gelfoam in the central well of an organ culture dish. Cultures were treated with 11, 13, and 15 μM/L concentration of Al maltolate for up to 24 days. Neurofilamentous aggregates were induced which were positive for the high and middle MW neurofilament subunits. No tau immunopositivity was seen but this must be placed in perspective, as many of the present day monoclonal antibodies were not available when this study was carried out (57).

In Griffioen *et al* (2004), studied whether in human teratocarcinoma (NT2) precursor cells, and found Al maltolate at a concentration of 500 μM caused significant cell death. Nuclear fragmentation suggestive of apoptosis was observed as early as 3 h and increased significantly through 24 h (54). TUNEL positive nuclei were also observed. The release of cytochrome *c* suggestive of mitochondrial injury was demonstrated by Western blot (WB) analysis. Savory *et al* (2006) studied the development of a neuronal culture system to evaluate the neurotoxic effects of Al maltolate on fetal rabbit midbrain sections containing the oculomotor nucleus (126).

Studies from our laboratory (64, 65, 67, 70, 71) on chronic Al exposure depicted a distribution of Al in different brain regions and body organs of male albino rats (wistar strain) weighing approximately 100–130 g. All animals received about 10 mg Al/kg bw/day, in the form of aluminum lactate dissolved in distilled water, intragastrically for 12 weeks. Distribution of Al in the different regions of brain (cerebral cortex, hippocampus, corpus striatum, cerebellum, and brain stem) revealed that, following subacute Al exposure, the maximum accumulation of Al occurred in the hippocampus followed by the corpus striatum, brain stem, cerebral cortex, and cerebellum whereas, following acute Al exposure, the maximum accumulation was in the corpus striatum followed by hippocampus, brain stem, cerebral cortex, and cerebellum (67, 71). With chronic (71) and subacute (67) exposure of aluminum to Wistar male rats, the maximum accumulation was in the hippocampus. There was almost a 24-fold increase in aluminum accumulation in the hippocampus following chronic aluminum exposure and 80-fold following subacute exposure as compared to control animals.

This result demonstrates the cumulative nature of Al in the hippocampus during subacute and chronic treatment. It can be reasoned, that excessive accumulation of Al in the hippocampus could result in loss of memory which is a characteristic feature of neurodegenerative diseases in which Al has been implicated.

Studies from our laboratory, on the subcellular localization of this metal ion, revealed that Al resides primarily in the nucleus of the cell (67). This finding suggests that Al may be more stably bound to the phosphate groups of DNA in the nuclear fraction rather than to the other cellular constituents. An appreciable amount of Al was also bound avidly to cytosolic, microsomes,and to mitochondria, which may be responsible for the inhibition of some of the mitochondrial functions such as cellular respiration, oxidative phosphorylation, and antioxidant enzyme activity (67).

Another study from our laboratory confirmed that maximum accumulation was in hippocampus, which exhibited more than 24-fold increase in Al levels, followed by corpus stratum fivefold) and finally cerebral cortex fourfold). In the same study, the accumulation of neurofilaments which are fundamental units of NFTs in cerebral cortex, corpus striatum, and hippocampus was also observed. This high molecular weight neurofilament protein (200 kDa) was identified and confirmed by immunoblotting using the antineurofilament [H] as the primary antibody (71) (Figure 1). These results were consistent with results by Leterrier *et al* (1992) (83).

Savory *et al* (2003) employed Al maltolate for intravenous administration to adult rabbits for 8–30 weeks. Although no

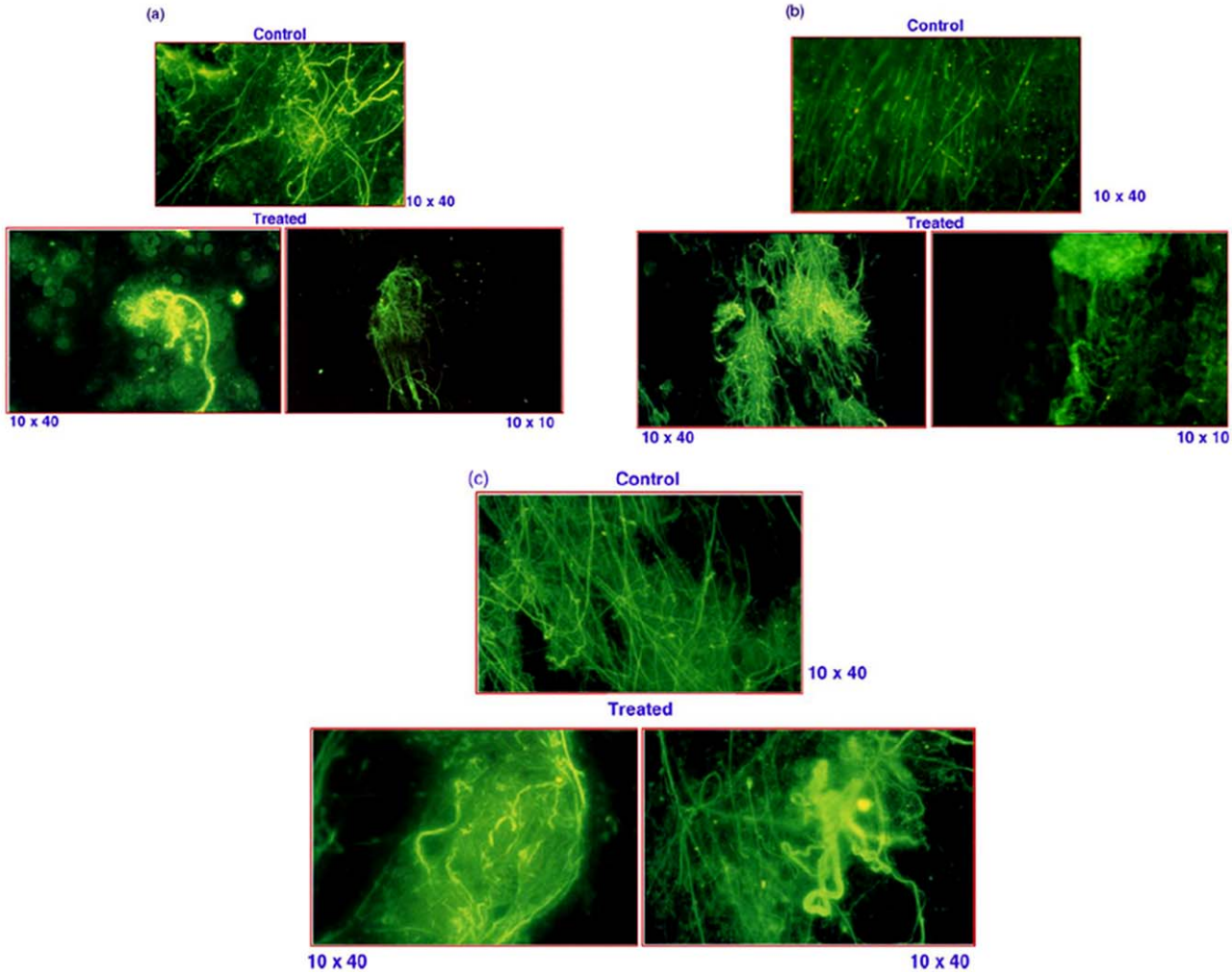

Figure 1. (**A**) Immunofluorescence of high molecular weight neurofilament in cerebral cortex, (**B**) immunofluorescence of high molecular weight. neurofilaments in corpus striatum, (**C**) immunofluorescence of high molecular weight neurofilaments in hippocampus (Male albino rats (Wistar strain) exposed to 10 mg/(kg bw day), aluminum for a period of 12 weeks, smears were prepared from the three neuronal regions studied earlier, that is, cerebral cortex, corpus striatum, and hippocampus. These were initially stained with antineurofilament anti body followed by staining with FITC-labeled secondary antibody. The slides were then observed under a fluorescence microscope) *Toxicology 219 (2006) 1–10*.

consistent neurological symptoms were observed in the Al-treated animals, weight loss were noted. A slight increase in brain tissue Al concentration was seen by an electrothermal atomic absorption spectrophotometric technique (125). Berthold *et al* (1989) observed neurofibrillary changes in the oculomotor nucleus in about one-third of the treated rabbits, as demonstrated by a monoclonal antibody to the 200 kDa subunit of neurofilament protein (7). More distinct evidence of neurofibrillary changes has been detected by Forrester and Yokel (1989) in a comparative study of subcutaneous and intraventricular administration of Al lactate to adult New Zealand white rabbits. For the subcutaneous study, rabbits received daily subcutaneous injections of 400 μmol/kg Al lactate over a period of 4 weeks. Although no consistent clinical pattern of neurotoxicity was apparent, two of the animals displayed some evidence of motor abnormalities which included foot spread on landing, loss of the righting reflex and gait abnormalities. Neurofibrillary changes, detected by the Bielschowsky's silver method, were evident in the hippocampus and frontal cortex of the affected animals (41).

In a later report aimed specifically at relating Al neurotoxicity to age, Yokel used the subcutaneous and intraventricular routes of Al exposure in older (2–3.4 years) New Zealand white rabbits. Because the older animals were more susceptible to the higher dose (400 μmol/kg) of Al lactate used in the younger animals, the dose was reduced to 200 μmol/kg. Tissue Al concentrations in the frontal cortex and hippocampus were found to be significantly elevated even at this lower dose of Al (161).

Mawal-Dewan *et al* (1996) reported NFTs, one of the histologic hallmarks of AD, using the rabbit model system and Al maltolate intracisternal injections, analyzed by light microscopy (95). Other pathological hallmarks of AD, such as neuritic plaques, are not observed in Al maltolate-induced encephalopathy. Intriguingly, they found an abundance of NFTs coupled to a relative paucity of

neuritic plaques characteristic of the ALS/parkinsonism-dementia complex of Guam, suggesting that widespread NFTs (without Aβ plaques or significant Aβ deposition) indicate neurodegeneration (95). In AD, neuritic plaques often show a perivascular predilection, suggesting that dystrophic neurites and accompanying amyloidogenic accumulation may be pathogenically linked to vascular or microvascular associated factors. Cerebrovascular pathology may be relevant in the pathogenesis of naturally occurring AD, and may account for increased Al accumulation during central nervous system damage. Conversely, in experimental Al-induced neurofibrillary degeneration, cerebrovascular pathology is not observed in the nonsenescent laboratory rabbit. In experimental neurodegeneration, the mode of Al delivery in the central nervous system is often direct, as with intracisternal, intraventricular, or intracerebral modes of administration, thereby, effectively bypassing the blood-brain barrier. This may be related to the type and distribution of neurofibrillary degeneration in rabbits which, in some respects, differs from the more naturally occurring NFTs in AD in humans (94, 95). Furthermore, the formation of neuritic plaques appears to be species dependent, being found in few mammalian species and then, only in the aged (126). As described below, these differences are less compelling when compared to the broad array of immunochemical similarities which exist between experimental Al-induced neurofibrillary degeneration in rabbits and the various neurofibrillary lesions of neurodegenerative disorders in humans. The relationship of Al-induced neurofibrillary degeneration in rabbits to the neuritic pathology in human neurodegenerative disorders has been viewed with scepticism, because of the apparent ultrastructural differences between the experimental lesions in rabbits and NFTs in humans. Compared to the tangles of AD, which mostly contain paired helical filaments, those produced by Al are predominantly straight (intermediate-like) filaments (74, 153–155).

Interestingly, tau has been observed to form Alzheimer's-like filaments *in vitro* (26, 104). Al-induced neurofibrillary degeneration is characterized predominantly by immunohistochemical staining with antibodies specific for epitopes of hyperphosphorylated neurofilament protein(60, 144). The primary constituent of paired helical filaments in human NFTs is tau in association with abnormally phosphorylated neurofilament proteins, ubiquitin, α1-antichymotrypsin, AβPP, and Aβ. Studies over the past several years (102, 135) have demonstrated that immunochemical similarities between the composition of the Al-induced lesions and those found in AD are far closer than was originally surmised. Using a variety of mAbs that recognize both nonphosphorylated and phosphorylated tau, namely Tau-1, Tau-2, AT8, PHF-1, and Alz-50 (102, 123), it has been observed that abnormally phosphorylated tau is present in these Al-induced neurofilamentous aggregates (60). Savory *et al* (1996) reported the time course of aggregation of these cytoskeletal proteins. The results indicate that the aggregates may become detectable by silver staining as early as 24 h following Al maltolate administration and those neurofilament proteins predominate. Nonphosphorylated/phosphorylation-independent epitopes, as detected by mAb SMI-33, are found first, followed by the phosphorylated forms, immunostained by mAb SMI 31, at approximately 72 h. Tau is also detectable by around 72 h, although the characteristic epitopes of AD as recognized by mAbs AT8 and PHF-1 are most distinct at 6–7 days following Al injection. It has been proposed that phosphorylation of cytoskeletal proteins drives the formation of the neurofilamentous aggregates, particularly in human neurodegenerative disorders (96). As these aggregates are hyperphosphorylated, phosphorylation alone would render these protein accumulations unstable, because of the preponderance of negative charges on the phosphate groups. Thus, it is reasonable to propose that some positively charged species constitute an inherent factor in the formation and stabilization of the neurofibrillary protein aggregates which are rich in phosphate groups and would have an extremely strong affinity for positively charged anions. This could occur in AD and in experimental Al-induced neurofibrillary degeneration; in the latter, Al^{3+} is an obvious candidate for this role. Savory *et al* (1996) have shown some features of Al-induced changes in rabbits using the intracisternal route of administration. This study demonstrates dramatic neurofibrillary changes using the Bielschowsky's silver impregnation technique on a frozen section of temporal cortex; these changes have many similarities at the light microscopic level to NFTs seen in humans. WB analysis of homogenized tissue is clearly positive for tau protein which is the major constituent of the NFTs seen in AD (123).

A recent report claimed that 100 μM Al maltolate induces tau aggregation *in vitro* (Mizoroki et al 2007), but lacks that same effect *in vivo* (100). Their research results provided evidence showing Al does not encourage the etiology of AD. Savory and Ghribi (119–121) and Exley (2007) criticized the methodology utilized by Mizoroki *et al* The response from Mizoroki et al has been to indicate that the absence of Al maltolate treatment is a proof for the claim that Al is not a causative agent in the etiology of AD (32). However, brain amyloidosis can be increased in AβPP transgenic mice through the use of dietary Al. Recently Mizoroki *et al* (2007) concluded that the failure of treatment of transgenic mice with Al maltolate to induce Alzheimer's-like neuropathological changes provides proof that Al is not a causative agent in the etiology of AD. Dietary Al has been shown to increase brain amyloidosis in AβPP transgenic mice (114). As NFTs and neuritic plaques appear to be seen in only a few animal species including aged bears (23) and, of course, humans, it is not surprising that Al compounds in most species fail to reproduce the neuropathological and biochemical abnormalities seen in AD. Such a lack of an exact match in abnormal features does not rule out this metal ion as a contributing factor in the etiology of AD.

Al should certainly be high on the list of possible candidates for contributing to AD. First, it is highly abundant in the environment, being the most abundant metal in the Earth's crust. Second, it is extremely neurotoxic once it gains access to the central nervous system. Its proven toxicity in dialysis encephalopathy attests to this neurotoxicity, as do many experimental animal studies (126). Studies using rabbits may be particularly relevant to the investigation of human disease as they belong to the mammalian order Lagomorpha, according to an impressive 88 protein sequences (77). The Mizoroki *et al* (2007) experiments have not tested the hypothesis that a physiologically significant concentration of Al could influence the aggregation of tau *in vivo*. Therefore, it is *unreasonable* for the authors to conclude that "these results indicate that Al has no direct link to AD pathology" without conducting further studies to test this hypothesis (100).

Al exposure and neurobehavioral changes

Based on earlier reports from our laboratory (64, 65, 67), it has been shown that following chronic and subacute exposure to Al, male wistar rats showed maximum accumulation of Al in hippocampus.

There were decreased number of ligand receptor binding sites (***B***max) observed in the hippocampus, cerebral cortex and corpus striatum of Al treated animals, with the effect being most pronounced in the hippocampus. From these reports, it can be concluded that hippocampus which is the seat of memory and learning is the most affected region of the brain. Decrease in the ***B***max value suggests that there is a decrease in the number of receptors in hippocampus, implying that a decreased transmission of nerve impulses may be responsible for neurobehavioral alterations (64, 65, 67). A similar decrease in the number of receptors can be responsible for the state of dementia, as observed in patients with AD (92). Our data also revealed a significant retardation in the retention of the learned task in the Al-exposed group compared to the control group. Hippocampal damage has been correlated with significantly decreased performance during memory function tests in experimental animals (64, 65, 67, 109, 113). The possibility that altered motor function could influence the changes in the memory tasks could be ruled out, as no significant changes in the spontaneous locomotor activity and rota rod tests were observed. Therefore, these tests can serve as an index of memory function.

A number of studies have been performed to evaluate the neurocognitive functions both in rodents as well as in humans, and many studies conclude that Al is potentially toxic to mammals even though Al neurotoxicity has been documented previously. This might be the case because neurobehavioral studies of Al in rodents have generally not produced consistent results. In one study testing neurocognitive function, rats exposed to Al were evaluated for motor activity in an open-field apparatus as well as for learning in a passive avoidance test. Al exposure presented no significant effects on behavior of the rats, although, exposure did result in a decrease in the total numbers of synapses with age (30). Golub *et al* (1992) exposed adult mice to Al, using a diet high in Al content (1000 micrograms Al/g diet) for 90 days. Following exposure, a decrease in motor activity, grip strength, and startle responsiveness in the mice was observed (51). The behavioral and neurochemical effects of Al exposure on mice gestation and offspring have been long documented (3); and now, it is well established that an excess of Al during gestation and lactation, at levels below maternal toxicity, can result in persistent neurobehavioral deficits in the offspring of rats and mice (51, 139). No deleterious effects of Al ingestion were found in cognitive behavior when 22-day old rats were given Al hydroxide for 60 days (139). However, studies on the neurobehavior effects of oral Al exposure in adult animals have shown contradictory results (11, 50). Similarly, no significant differences between control and Al-treated rats were found in behavioral tests for short and long-term memory (18). Although, on the contrary, Connor *et al* (1988, 1989) observed impaired performance of rats in a passive avoidance task after administration of Al sulfate in drinking water for 1 month. The passive avoidance conditioning test results were similar to previously reported findings (27, 28), with no significant effects of Al on this parameter. Recently, Li *et al* (2006) have reported cognitive dysfunction in mice exposed to Al chloride and suggested that an increase of dissociated Ca^{2+} in hippocampus neurons may be a possible mechanism for cognitive impairment (85).

Al induced symptoms mimic AD symptoms

The neurotoxic symptoms resulting from Al exposure include speech disturbances, dyspraxia, tremors, partial paralysis, and marked decline in learning and memory. Additionally, Al is also linked to other pathologies such as dialysis dementia, microcytic anemia, osteomalacia, and epilepsy (30). Our laboratory studies (66, 71) have also shown that Al exposure causes neuropathological (Figure 1), neurobehavioral, and neurochemical changes resulting in impaired learning ability.

Mounting evidence in recent years has suggested that Al has severe toxic manifestations on the central nervous system. Al causes changes in acetylcholinesterase activity from acute and subacute exposure as well as significant deterioration in cognitive functions in rats (21, 66, 78, 108, 166). Observation of the central cholinergic system after Al exposure, of 10 mg/kg of body weight/day for 4 weeks, revealed a deleterious effect on the activities of biosynthetic (choline acetyltransferase) and hydrolytic (acetylcholinesterase) enzymes of the neurotransmitter acetylcholine. Following the dose regimen, the levels of acetylcholine were significantly lowered in different brain regions and there was a significant decrease in high-affinity choline uptake. Muscarinic acetylcholine receptor binding studies have revealed a decreased number of binding sites, with the maximum effect manifesting in the hippocampus. Exogenous addition of 10 μM desferrioxamine restored the muscarinic receptor binding completely. Impaired cholinergic functioning results in severe effects on cognitive functions, manifesting as neurobehavioral deficits shown by decreased performance in active (52%) and passive (73.30%) avoidance tests. The neurobehavioral deficits observed in these studies suggest that Al is toxic and is altering cholinergic neurotransmission (66).

Recently, Ribes *et al* (2008) have indicated that chronic low level exposure to Al lactate may lead to disrupted spatial learning and neurogenesis in a transgenic mouse model of AD. In this study, from 5 months age onwards they fed wild type and APP-Tg 2576 mice with diets supplemented with Al lactate at 0 and 1 mg/g of diet for 120 days. The activity in an open-field and learning in a water maze were evaluated after 3 months of Al exposure. Transgenic mice presented with impairments to both acquisition and retention of the water maze task. These mice also showed higher amounts of Aβ fragments in the brain. Al exposure impaired learning and memory in mice and increased the total number of proliferating cells in the dentate gyrus of hippocampus. The authors suggested that low Al doses might impair cognition in the general population at doses comparable to current levels of human exposure (115).

Another chronic study with Al gluconate showed behavioral alterations such as disturbed exploratory behavior, catatonic behavior, and emotional reactivity alterations as well as brain modifications such as decrease in neuronal density in the parietal and temporal cortex andinvaginated nuclei with intense eosinophilic cytoplasm in the hippocampus, temporal, and parietal cortex. Experimental adult rats that exhibited significantly lower performance scores in the shuttle-box task along with lower and fluctuating performance scores indicative of impaired spatial memory in the Morris water maze were found to have hippocampal neurons and cortical neurons with chromatin clumping and vacuolations in the nucleus and cytoplasm (98). The Miu authors have also demonstrated decreased scores of activity and emotionality in Al exposed, aging animals along with cellular and ultrastructural degenerative signs by electron-microscopic analysis (99). Jing *et al* (2004) performed similar experiments that corroborated the conclusion that rats exposed to Al present learning and memory impairments (63).

The reports of neurocognitive dysfunctions have also been published on humans. The relationship between an elevated body Al burden and central nervous system function was studied in 25 current mild steel welders and 65 aluminum welders and body burden was estimated. They analyzed Al concentrations both in serum (S-Al) as well as in urine (U-Al) with graphite furnace atomic absorption spectrometry with Zeeman background correction. Referents, low-exposure and high-exposure groups were defined according to an aggregated measure of aluminum body burden, the group median S-A1 levels being 0.08, 0.14, and 0.46 μmol/L, respectively, and the corresponding values for U-A1 being 0.4, 1.8, and 7.1 μmol/L (119). The subjects presented symptoms such as increase in fatigue, mild depression, and memory and concentration problems. Neuropsychological testing revealed adverse effects of Al in attention related tasks and the working memory system. Giorgianni *et al* (2003) also studied cognitive disorders among welders exposed to Al and concluded that workers exposed to Al presented impairment in memory and concentration (49). Some other epidemiological studies also showed poorer performance in cognitive tests or a higher level of neurological symptoms for workers occupationally exposed to Al (117, 136). In another study, Kiesswetter *et al* (2009) performed a longitudinal study, over 4 years, on potential neurotoxic effects of Al and assessed the association of Al exposure and neurobehavioral performance of Al welders in the train and truck construction industry (75). The biomonitoring data of the welders showed a high long-term stability but also presented with sensitivity to acute shift dependent exposure changes. Al welders working in this profession an average of 15 years showed no significantly increased symptom levels compared to the control groupExplorative regression and covariance analyses revealed neither a correlation between biomonitoring and performance variables nor a significant difference between Al exposed and controls in the performance courses during the 4 year period (75). In their first longitudinalstudies, recorded neurobehavioral health and Al exposure of welders in the train and truck construction industry over 4 years, with intermediate results after 2 years (15), that showed that welders in this industrial sector were exposed to considerably higher levels of Al (90–150 lg Al/g creatinine in urine) than welders of a parallel study in the automobile industry (30–40 lg Al/g creatinine in urine) (76).

Recently, Lu et al (2014) studied Tau-protein expression and cognitive disorders in sixty six (88) retired Al smelting workers. The cognitive functions were assessed with the Mini Mental State Examination. The tau-protein expression in peripheral blood lymphocytes was analyzed with western blots. The cognitive functions of the exposed group were significantly decreased. Twelve mild cognitive impairment cases in the exposed group and four mild cognitive impairment cases in the control group were diagnosed. Significantly higher p-tau181 and p-tau231 levels were detected in the Al-exposed workers than in the control. They suggested, that long-term exposure to Al may cause cognitive disorders (88).

Many, but not all, publications to date have shown cognitive impairments and/or neurobehavioral changes following Al exposure. While many studies reported neurobehavioral changes, other studies not showing lack of Al exposure-related deterioration of performance has led some to question the diversity of results (2, 15, 61, 75, 76). Taken together, these conflicting findings on learning impairments may be attributed to the differences in duration of dose and route of exposure. More studies of occupational Al exposure need to be conducted with a broader range of concentrations (doses), different routes, and varying durations of exposure to Al to corroborate the conditions needed to demonstrate Al neurotoxicity in humans. Studies that show a positive effect of Al on cognitive performances should be explained by studies that promote a negative findings, just as positive studies attempts to explain the occasional study with negative results.

MECHANISM OF AL TOXICITY IN RELATION TO AD

The exact mechanism by which Al crosses the blood brain barrier (BBB) is unknown. Most of the studies that have examined the effects of acute injections of Al have reported that it does not disrupt the BBB or alter cerebral blood flow. However, because Al and iron possess similar properties, Al is thought to cross the BBB using the same mechanism as iron, namely in transferrin receptor (68).

Additionally, Al has been shown to increase the rate of transmembrane diffusion through the BBB and to selectively change saturable transport systems without disrupting the integrity of the membranes or altering the hemodynamics of the central nervous system and causing pathological features of AD. Hence, to understand the toxicity of Al, identification of an appropriate Al compound which can easily diffuse through BBB is immensely important. The electroneutral Al-maltolate appears ideal as this compound can deliver a significant amount of free aqueous Al at physiological *p*H. Unlike most other Al salts, such as $AlCl_3$, it produces insoluble complexes at neutral *p*H. Al-maltolate increases the soluble Al concentration from 4–6 mM compared to other organic Al salts like Al-lactate or Al-aspartate (soluble Al concentration is ~55–330 μM). Al-maltolate is soluble and stable from pH 3.0 to 10.0, while hydrolytically stable at **pH 7.0, and has no speciation chemistry problems. Al-maltolate is suitable over other Al compounds because of its very high metal solubility at *p*H 7.0 and prominent kinetic restrictions to ligand exchange reactions in neutral solution. Based on our own contributions, Bharathi *et al* (2003), Liang et al (2012), Kaneko et al (2006), Wang et al (2014), and those of Savory *et al* (1996, 1999, 2003, 2006), it is understood that Al-maltolate is a suitable compound for toxicological and neuropathological studies in relevance to AD (8, 69, 87, 123–127, 150).

Al is a neurotoxic element and there are various proposed mechanisms for its neurotoxicity including: impaired glucose metabolism, free radical damage via increased lipid peroxidation, protein modification, consequences on signal transduction, disturbances in the axonal transport, and altered phosphorylation of neurofilaments (71). Some studies have reported that Al kills individual neurons secondary to intracellular accumulation and that cell death is not secondary to the generation of reactive oxygen species nor the accumulation of intracellular calcium even though Al causes an elevation in both (66, 71). Several studies have explored the role of Al induced oxidative stress via free radical generation in the early onset or evolution of AD in transgenic models. The most notable feature in the oxidative stress hypothesis for AD and other neurodegenerative diseases is that cumulative oxidative damage over time could account for the late life onset and slowly progressive nature of AD and Parkinson's disease (PD) (24).

Evidence for increased oxidative stress in AD includes: increased levels of elements capable of initiating free radical

formation such as Al, mercury, and iron in brain (17); increased protein oxidation; increased DNA oxidation; increased lipid peroxidation and decreased polyunsaturated fatty acids in AD brain; and the ability of Aβ to generate free radicals (17). Pratico *et al* (2002) reported that chronic dietary administration of Al can increase Aβ levels and accelerate plaque deposition in an animal model of AD-like amyloidosis. Increase in the levels of specific and sensitive markers of oxidative stress *in vivo* correlated with increased levels of Aβ and was consistent with an exacerbation of brain lipid peroxidation (114).

Al promotes phosphorylation of the tau protein (133), which results in it being less soluble, increasing the likelihood that the protein will aggregate. Tau protein is the microtubular associated protein that accumulates in NFTs. By targeting tau, Al may act to promote the development of NFTs. This result has led some to suggest that AD is a result of hypoactive phosphatases and overactive kinases (47, 122). There is also evidence that Al may alter the dynamics of Aβ (4). The core center of amyloid plaques is known to contain an overabundance of Aβ42, which is less soluble than the more abundant Aβ40. This alone suggests the possibility that abnormal cleavage of AβPP may play a role in the development of AD. However, there is now clear evidence that when Al complexes with $A\beta_{42}$, it reduces solubility, increases precipitation of β-sheets, and facilitates Aβ flux across the BBB (31).

In spite of all of the evidence for a central role of Aβ (especially from genetic studies) and tau protein in AD, there remains some reason to question whether the abnormalities in these proteins are causative of the disease or are only reflections of the true underlying etiologic factor (107). Bancher *et al* (1997) showed that while neurons in physical contact with an amyloid deposit or containing a NFT were more likely to die than those neurons which did not, most dying cells were not located within amyloid deposits and most dying cells did not bear a tangle, nor were they near to a neuron with a tangle. Although the precipitated forms of Aβ and tau protein may not be physiologically active, there certainly remains the possibility that circulating or intracellular forms of these proteins, perhaps forming complexes with Al, are toxic, but other primary causes of neuronal cell death cannot be excluded (4).

Role of Al on AβPP processing in the formation of Aβ

Another finding in Al-induced neurodegeneration has been the observation of increased levels of AβPP and Aβ in neurons of Al treated rats (132) and rabbits (60). NFTs in AD also exhibit this Aβ staining but the most prevalent pattern is the presence of Aβ in the neuritic plaques. It is logical to hypothesize that in AD, increased Aβ initially appears intracellularly, followed by extracellular deposition after cell death. Other works (59, 110, 164) have described an Al-induced secondary structural transition in the non-Aβ component of AD amyloid (NACP), generating approximately a 33% α-helix, which renders NACP resistant to proteases. Based on this finding, it is suggested that Al may influence NACP protein turnover and induce aggregation via structural modifications thus leading to Aβ deposition (113). A microprobe method for detecting Al within plaques would have to be extremely sensitive. This required sensitivity is because Aβ has a molecular weight of 5000 kDa and Al(III) is trivalent (Al^{3+}) with an atomic weight of 27. This means it is possible that the ratio of Aβ to Al on a weight to weight basis could be 550:1.

Candy *et al* (1986) reported the presence of Al within plaques in the brain of an AD patient (16). Landsberg *et al* (1992) failed to confirm this finding with the alternative microanalytical technique of particle-induced X-ray emission (PIXE) using a nuclear microscopy; however, this nuclear microscopic technique is relatively insensitive below 15 μg/g of Al, making the significance of the report questionable (82). The nuclear microscope has yet to be validated for Al detection in biological tissue.

While the formation of NFTs is most likely a product and not a cause of neuronal death, Aβ, the principal component of the senile plaques (SP) characteristic of AD, has been shown to be neurotoxic, possibly because it traps excess toxic metal ions. Al is a constituent of SP and influences the aggregation and toxicity of Aβ (36). If Aβ is involved in neuronal cell death in AD, then the presence of Al in brain interstitial fluid (BIF) could mediate its action. This mediation may even involve Al-ATP which has been shown to promote amyloidosis of Aβ. It is not clear whether Al is also involved in the secretion into BIF of superphysiological concentrations of Aβ following cleavage of its precursor protein (AβPP) by a β-secretase. However, Al has been shown to promote aggregation of Aβ *in vitro* and accumulation of AβPP in neurons and glia (36).

The function of AβPP is not fully understood, and it has been shown to be neuroprotective. When cleaved by α-secretase AβPP releases a soluble fragment, sAβPP, which suppresses elevation of intracellular Ca^{2+} by reducing glutamate-induced NMDA currents (36). The secretion of sAβPP is regulated glutamaterigically through phosphatidyl-inositol-linked receptors. The potentiation of neuronal excitotoxicity by Al-ATP would be expected to result in the increased secretion of sAβPP. However, the co-localization of Al and AβPP in the Golgi complex and irreversible accumulation of AβPP induced by Al in neurons and glia (36) could suggest that AβPP binding of intraneuronal Al may prevent the release of sAβPP and, therefore, the amelioration of excitotoxicity. This could also be indirect evidence of an influence of Al on AβPP processing (84, 89).

The presence of Al in the cores of senile plaques has been controversial for a long time (17, 31, 62, 140). One of the reasons in these studies may be that Al in amyloid fibers, in the core, could not be stained histochemically while Al in the NFTs and nuclei of nerve cells in the AD brain is stained clearly by histochemistry (104, 134, 145). If Al is lightly dispersed in a tissue or is present in small amounts, the conditions are less than favorable for Al staining at the level of light microscopy. It seems likely that Al does not attach to the surface of amyloid fibers in the core after the aggregation of Aβ peptides but instead, that Al binds just before, and during, the course of Aβ peptide aggregation. It is widely believed that amyloid-Al complex are more toxic than Al or amyloid on their own and consequently play a key role in the etiology of AD (29). However, Aβ plaques are not necessarily toxic, given that Wujek JR *et al* (1996) reported that cells can readily grow on amyloid plaque removed from AD brain tissue (159). Amyloid fibers in the core have been reported to consist mainly of the aggregates of Aβ peptides that have a beta-sheet structure (129, 141). Recently Yumoto *et al* (2009) examined the presence of Al at autopsy of five AD patients using energy-dispersive X-ray spectroscopy combined with transmission electron microscopy (TEM-EDX). TEM-EDX analysis allows simultaneous imaging of subcellular

structures with high spatial resolution and analysis of small quantities of elements contained in the same subcellular structures. The Yumoto study, demonstrated colocalization of Al and Aβ peptides in amyloid fibers in the cores of senile plaques (165).

The results support the following possibilities in the brains of patients with AD: there is evidence for Al could be involved in the aggregation of Aβ peptides to form toxic fibrils (31). Al induction of Aβ peptides into the beta-sheet structure (35); and Al might facilitate iron-mediated oxidative reactions, which cause severe damage to brain tissues (10, 55). First, Al aggregation of Aβ peptides *in vivo* is probable because Al (Al^{3+}) is a trivalent cation, Al can interact with acidic groups of the peptides and bind these peptides to one another. It has been reported that Al ions promote aggregation of physiological concentrations of Aβ peptides *in vitro* (89). House *et al* (2004) and Khan *et al* (2005) reported that Al ions accelerate the formation of amyloid fibrils *in vitro* (58, 73). Second, Al causes the conformational change of Aβ peptides into the beta-sheet structure *in vivo*. Ricchelli *et al* (2005) reported that Al induces structural modification of Aβ peptides enriched in beta-sheet conformations *in vitro* (116). Exley (2006) pointed out that only Al and Iron (Fe), but not copper (Cu) or zinc (Zn), are involved in the formation of the beta-sheet structure of Aβ peptides (31). It has been proposed that Aβ peptides containing the beta-sheet structure are directly incorporated into membranes, forming Ca-permeable ion channels, and causing elevation of intracellular calcium levels that lead to nerve cell death. Third, Al promotes oxidative injury to brain tissues both on its own and by facilitating Fe-mediated oxidation reactions in the AD brain (31). Fe is involved in the formation of free hydroxyl radicals via Fenton chemistry, which may cause deleterious effects in brain tissues (31). It has been reported that Fe accumulates in the cores of senile plaques and that deposition of Aβ peptides facilitates the generation of reactive oxygen species in the presence of Fe *in vitro* (19, 20, 22, 137). Xie *et al* (1996) reported that Al can facilitate Fe-mediated oxidative injury in cultured neurons (160). In addition, Khan *et al* (2005) reported that Al potentiates the Fe(II)/Fe(III) redox cycle in favor of Fe(II) *in vitro* (73). These authors suggested that oxidative damage in the vicinity of senile plaques may be the result of Fenton reactions catalyzed by the co-deposition of Aβ peptides with Fe and Al. Based on these studies, it can be concluded that Al is linked to the generation of Aβ neurotoxic species.

Al inducesapoptosis, mitochondrial dysfunction, and endoplasmic reticulum stress

The hallmark of most neurodegenerative diseases is loss of neurons and there is mounting evidence that indicates apoptosis may be a significant mechanism for neuronal death in neurodegenerative diseases. Recently, our laboratory results have shown that chronic Al exposure increases ROS generation which is implicated in the impairment of mitochondrial functions in various regions of rat brain (79). All animals received Al about 10 mg/kg bw day, in the form of aluminum lactate dissolved in distilled water, intragastrically for 12 weeks. Following chronic Al exposure, there was significant decrease in the activity of mitochondrial electron transport chain complexes, resulting in reduced ATP synthesis. The levels of various mitochondrial cytochromes were also decreased. Additionally, there was increased oxidative damage to mitochondrial proteins, revealed by increased protein carbonylation and decrease in the activity of mitochondrial aconitase (79). This oxidative damage to mitochondria was reflected morphologically in electron microscopic studies which revealed changes in mitochondrial shape and loss of cristae (80, 81). Savory *et al* (1999) reported that aging is an important factor in the susceptibility of neurons to oxidative stress and subsequent apoptosis; both processes are observed in AD brain (127).

Mitochondrial dysfunction has been implicated in many neurodegenerative diseases; namely, ischemia-reperfusion injury, aging, and inflammatory diseases (152). Mitochondria are more prone to reactive oxygen species (ROS) induced cellular injury (86) as they lack protective proteins. Earlier reports from our lab have shown that chronic Al exposure results in decreased activity of cytochrome oxidase (COX) complexes I, II, and IV of the electron transport chain and to excessive production of ROS (79). Electron transport chain (ETC) dysfunction may ensue from mitochondrial damage including oxidation of mitochondrial DNA, proteins, and lipids, in turn leading to the opening of the mitochondrial permeability transition pore (MTP), an event associated with neuronal cell death (56). Enhanced ROS production and oxidative injury have been shown to play a cardinal role in the onset and progression of neurodegenerative disorders (46, 48, 128, 146). Defects in mitochondrial respiratory enzymes have been reported in several neurodegenerative diseases. Decreased complex I activity is reported in the substantia nigra of post-mortem samples obtained from neurodegenerative disease patients (128). Decreased mRNA levels of the mitochondrial-encoded COX subunits I, II, and III have also been known to occur in brains of AD patients (131). Reduced expression of nuclear encoded subunits of mitochondrial enzymes of oxidative phosphorylation (OXPHOS), including subunit IV of COX and the β-subunit of the F_1F_0-ATP synthase is also observed in vulnerable areas of AD brains (13).

Mitochondrial changes following cytotoxic stimuli represent a primary event in apoptotic cell death. Al has been demonstrated to accumulate in neurons following cell depolarization. Al inhibits Na^+/Ca^{2+} exchange and thereby induces an excessive accumulation of mitochondrial Ca^{2+} (138) which leads to opening of the mitochondrial permeability transition pore (MTP), with cytochrome *c* release and, subsequently, apoptosis by activation of the caspase family of proteases. Release of cytochrome *c* from the mitochondria into the cytoplasm has been shown to involve three distinct pathways. One implicates the opening of the MTP, the second involves translocation of the proapoptogenic Bax to mitochondria which can form a channel by itself, and the third apparently results from the interaction of Bax with the voltage-dependent anion channel (VDAC) to form a larger channel which is permeable to cytochrome *c*. On the contrary, the antiapoptotic regulator, Bcl-2, has the ability to block the release of cytochrome *c* from mitochondria by mechanisms such as a direct blockade of the MTP opening or by functioning as a docking protein (1). Al induced cytochrome *c* release is markedly reduced by cyclosporin A, a specific inhibitor of the MTP opening, and implicates opening of the MTP as the process by which cytochrome *c* translocates from mitochondria to the cytoplasm (1).

The endoplasmic reticulum (ER) is a multifaceted organelle that regulates protein synthesis, protein folding and trafficking, cellular responses to stress, and intracellular Ca^{2+} levels (93). Disruption of Ca^{2+} homeostasis, inhibition of protein N-linked glycosylation, expression of mutant proteins, and various other types of cellular

stresses cause accumulation of misfolded proteins in the ER lumen, resulting in ER stress (97). Al maltolate and $A\beta_{42}$ induce stress in the ER of rabbit brain. In response to the induced stress, the ER-resident protein, *gadd* 153, and the inducible transcriptional factor, NF-kB, are both translocated into the nucleus. Furthermore, this ER stress elicits an adaptive response triggering a signaling pathway called the unfolded protein response (UPR), which alleviates stress by induction of ER-localized chaperones, initiation of a degradation system, and attenuation of protein synthesis. However, if the stress is prolonged, it may lead to processing of the ER-resident protease, procaspase-12, as well as activation of calpain, caspase-3, caspase-6, and apoptosis. Although mitochondrial apoptotic signals are important in regulating apoptosis, the ER and nuclear organelles may also participate in the molecular mechanisms of apoptosis following neurotoxic stimuli. Recent evidence suggests that ER stress-mediated apoptotic signal pathways may contribute to the pathogenesis of AD (105). However, the precise molecular mechanisms underlying mitochondrial dysfunction and ER stress in neuronal cell death and neurodegeneration remain unknown.

Al appears to have a significant role in the induction of apoptosis, mitochondrial dysfunction, and ER stress in neurons and this induction is an important step to understand while moving toward AD neurodegeneration and pathogenesis.

CONCLUDING REMARKS

Plausible explanations for chronic Al intake as the cause of AD have been presented.

Many studies have revealed the neurotoxic role of Al in spatial learning, long-term potentiation and unaltered neurogenesis mechanisms, cellular respiration mechanisms through ETC chain and TCA cycle, cell death mechanisms through apoptosis, inflammatory response through NF-κB mechanism, antioxidant activity through SOD, and, of particular note in this review, in the formation of the hallmarks of AD such as senile plaques and NFT formation (Figures 2 and 3). In most of the studies reviewed here, the routes of Al exposure were through intragastric, oral, intracisternal, intraventricular, or intracerebral modes of administration and are effectively bypassed the blood-brain barrier (BBB). Oral studies are very good because they mimic human Al exposure risk, but because only a limited amount of Al is absorbed, it necessary to have a much longer exposure periods for Al to accumulate in neurons to toxic amounts. AD has a long prodromal phase, with pathologic changes often preceding the onset of clinical symptoms by more than a decade. High Al absorption may someday be recognized as a risk factor for AD. Humans usually are exposed to Al neurotoxicity either through the skin or through food additives. For

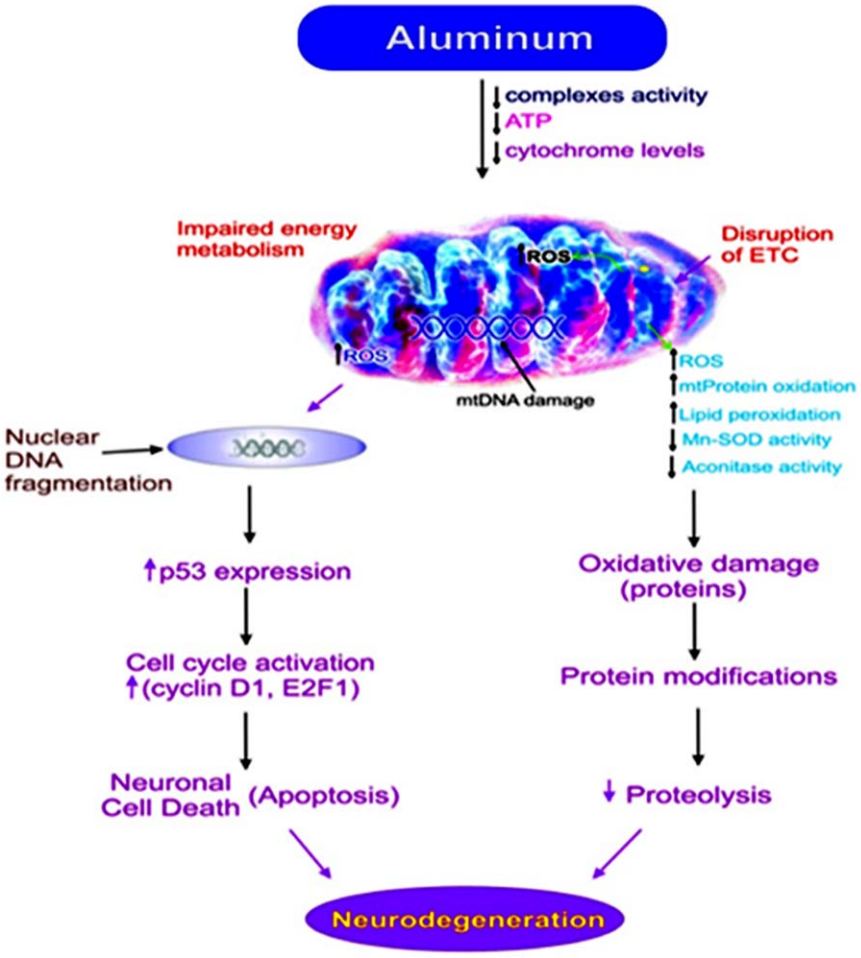

Figure 2. Possible Mechanisms for the role of Aluminum in the pathogenesis of Neurodegeneration via mitochondria (mt).

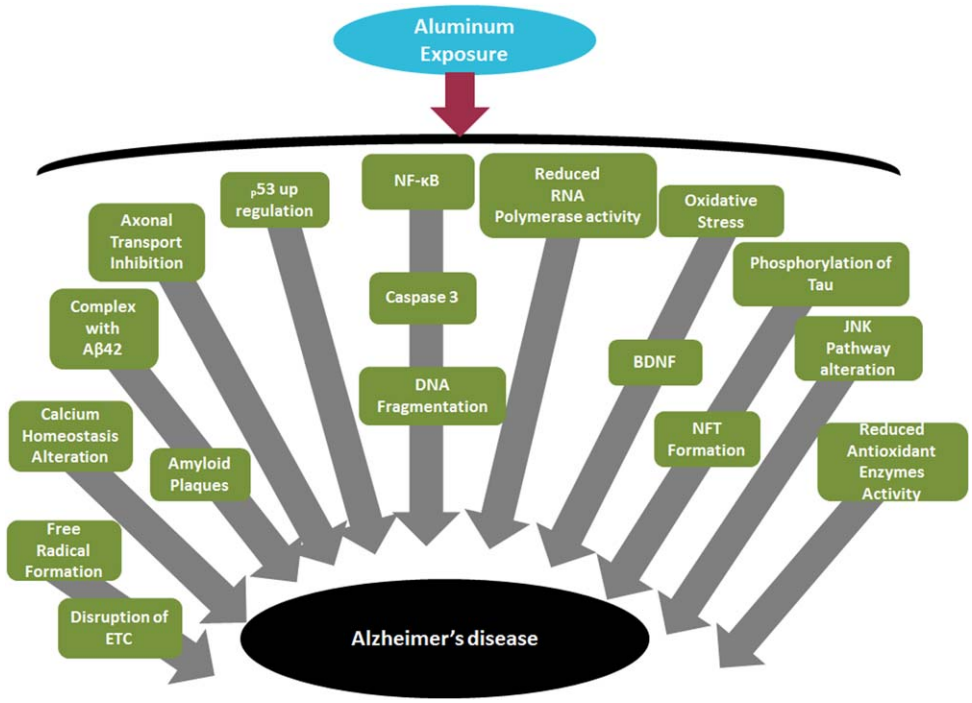

Figure 3. Hypothetical model for aluminum neurotoxicity in neurodegeneration—depicts a general overview of damage and disrupted regulatory pathways leading to AD.

this reason, it is crucial to do more research on the bio-availability of this metal and its effectiveness in crossing the gastrointestinal and the blood brain barriers, which could show prime cellular changes in the pathogenesis of AD.

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